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The progress and future of enhancing antiviral capacity by transgenic technology in the silkworm *Bombyx mori*

Liang Jiang, Qingyou Xia*

State Key Laboratory of Silkworm Genome Biology, Southwest University, Chongqing 400715, PR China

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ABSTRACT

Bombyx mori is a common lepidopteran model and an important economic insect for silk production. *B. mori* nucleopolyhedrovirus (BmNPV) is a typical pathogenic baculovirus that causes serious economic losses in sericulture. *B. mori* and BmNPV are a model of insect host and pathogen interaction including invasion of the host by the pathogen, host response, and enhancement of host resistance. The antiviral capacity of silkworms can be improved by transgenic technology such as overexpression of an endogenous or exogenous antiviral gene, RNA interference of the BmNPV gene, or regulation of the immune pathway to inhibit BmNPV at different stages of infection. Antiviral capacity could be further increased by combining different methods. We discuss the future of an antiviral strategy in silkworm, including possible improvement of anti-BmNPV, the feasibility of constructing transgenic silkworms with resistance to multiple viruses, and the safety of transgenic silkworms. The silkworm model could provide a reference for disease control in other organisms.

1. Introduction

Sericulture is a principal source of income for farmers in many developing countries such as China, India, Brazil, Vietnam and Thailand. Cocoon production by China is almost 80% of worldwide production. In 2011, China produced 6.61×10^8 kg of cocoons; the income of sericulturists was 22.4 billion Yuan and the value of the silk industry output was 203.8 billion Yuan.

Sericulture faces biological challenges from pathogenic viruses, fungi and bacteria, which cause losses of almost 20% of potential cocoon production each year (Jiang et al., 2013c). Viral diseases are responsible for almost 80% of total cocoon loss. These diseases are induced mainly by *Bombyx mori* nucleopolyhedrovirus (BmNPV) (Gomi et al., 1999; Rahman and Gopinathan, 2004), *B. mori* cytoplasmic polyhedrosis virus (BmCPV) (Cao et al., 2012) or *B. mori* densovirus (BmDNV) (Tijssen and Bergoin, 1995; Wang et al., 2007). BmNPV is the most prevalent threat to sericulture in almost all countries.

BmNPV, a member of the Baculoviridae family, has a circular double-stranded DNA genome (Gomi et al., 1999) that combines with capsid proteins to form a nucleocapsid that is contained within an envelope (Kondo and Maeda, 1991). The NPV replication cycle has two virion phenotypes: occlusion-derived virus (ODV) is transmitted among hosts and budded virus (BV) spreads throughout the host (Keddie et al., 1989; Rahman and Gopinathan, 2004). ODV but not BV virions are packaged and protected in a polyhedral body that is a highly symmetrical, covalently cross-linked lattice (Ji et al., 2010). BmNPV invades silkworm larvae mainly via oral infection. Polyhedral bodies are dissociated and ODVs are released in the alkaline environment of the gut juice after ingestion (Horton and Burand, 1993; Keddie et al., 1989). The peritrophic membrane is destroyed by the virus, creating holes that facilitate the passage of ODVs (Wang and Granados, 1997). Nucleocapsids enter the columnar epithelial cells of the midgut by envelope-mediated membrane fusion to initiate primary infection (Horton and Burand, 1993; Keddie et al., 1989). Viral DNA is released from nucleocapsids to be used as a template to generate new DNA and mRNA (Horton and Burand, 1993; Keddie et al., 1989). Viral proteins are synthesized using host components. Subsequently, progeny nucleocapsid obtains an envelope by budding from the host cell membrane to generate a BV that causes secondary infection via the host tracheal system (Engelhard et al., 1994; Slack and Arif, 2007). At the late stage of infection, progeny ODVs are assembled into polyhedral bodies that are released into the environment after host disintegration (Horton and Burand, 1993; Keddie et al., 1989; Slack and Arif, 2007) (Fig. 1).

Breeding resistant strains by traditional or transgenic methods is an approach to silkworm disease control. Disease resistance and

* Corresponding author. Tel.: +86 23 68250099; fax: +86 23 68251128.
E-mail addresses: jiangliang@swu.edu.cn (L. Jiang), xiaqy@swu.edu.cn (Q. Xia).

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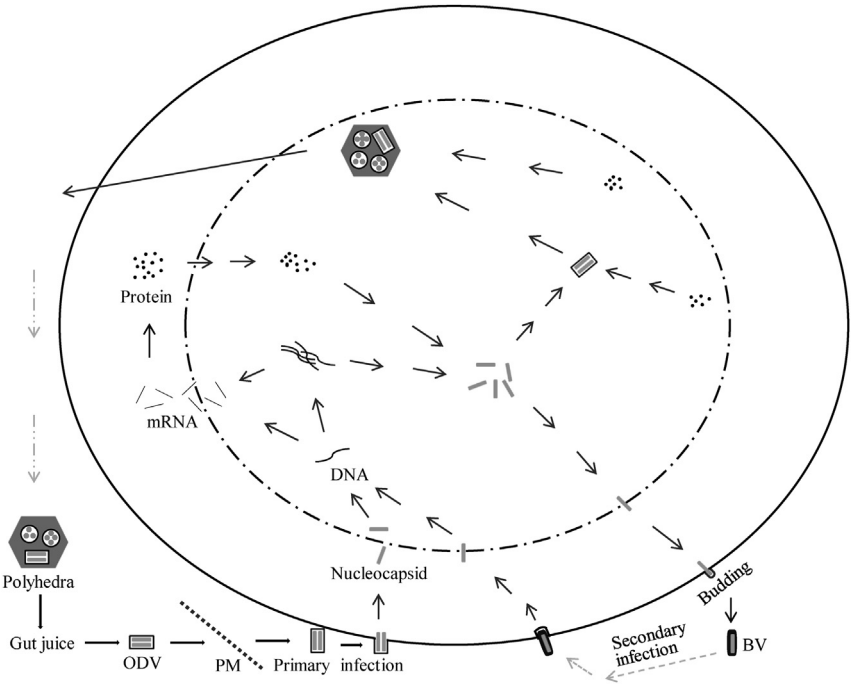

Fig. 1. The BmNPV infection process. Occlusion-derived virus (ODV) is released from polyhedra in the alkaline environment of insect gut juice after ingestion. The virus passes through the peritrophic membrane (PM) and nucleocapsids enter midgut columnar epithelial cells by envelope-mediated membrane fusion to initiate primary infection synthesis. Viral DNA is released from nucleocapsid and used as a template for DNA and mRNA. Replicated DNA and synthesized proteins assemble into progeny nucleocapsids that obtain an envelope from the host cell membrane by budding. Subsequently, budded viruses (BVs) spread through the host using the tracheal system to cause secondary infection. At late-stage infection, progeny ODVs are assembled into polyhedral bodies that are released after host disintegration.

economic characteristics are the two most important traits in breeding silkworm strains. Traditional breeding methods have limitations such as enhancing pathogen resistance at the expense of the quality of economically important characteristics (Jiang et al., 2012a). To date, a few resistant silkworm strains have been bred by traditional methods and none have been applied in sericulture. The limitations of traditional breeding methods might be avoided by transgenic technology, which theoretically changes only the target trait. Overexpression and RNA interference (RNAi) are two established gene regulation strategies that have been applied in some organisms to improve pathogen resistance.

B. mori, a lepidopteran model (Duan et al., 2010; Mita et al., 2004; Xia et al., 2007, 2008, 2004, 2009) and BmNPV, a typical baculovirus (Gomi et al., 1999), are a model of insect host and pathogen interaction. Studies of viral genes (Gomi et al., 1999), the BmNPV invasion process (Rahman and Gopinathan, 2004), the silkworm immune response (Sagisaka et al., 2010; Xue et al., 2012), host antivirus genes (Nakazawa et al., 2004; Ponnuvel et al., 2003) and silkworm genomes (Mita et al., 2004; Xia et al., 2009, 2008, 2004) paved the way for developing a transgenic silkworm with antiviral properties. Enhancement of antiviral capacity by transgenic technology in the silkworm has important theoretical and practical values and could promote antiviral research in other animals and breeding antiviral silkworms for sericulture.

Antiviral research is pursued worldwide; for example the Nagaraju group (Kanginakudru et al., 2007; Subbaiah et al., 2013) have used transgenic technology to develop viral resistance in silkworms and created a transgenic silkworm with high resistance to BmNPV. However, problems that remain to be solved include further enhancing the anti-BmNPV trait and determining if a single major silkworm gene is responsible for resistance to BmNPV. In this review we explore the possibility of (1) creating transgenic silkworms with strong resistance to multiple viruses; (2) selecting silkworm strains for transgenic improvement; and (3) establishing the safety of transgenic silkworms. We pay particular attention to antiviral strategies based on the infection process of BmNPV, the future for antiviral improvement of silkworms, and challenges to commercial application of transgenic silkworms.

2. Silkworm immune defense and BmNPV resistance genes

Different silkworm strains have different resistance levels to BmNPV. The *B. mori* KN (NB) strain, which is highly resistant to BmNPV infection, and the 306 strain, which is susceptible (Bao et al., 2009; Qin et al., 2012), were used to investigate candidate resistance genes using different methods. Suppression subtractive hybridization revealed 8 genes (*gloverin-4*, *gloverin-3*, *lebocin*, *serpin-5*, *arylphorin*, *promoting protein*, *cathepsin B* and *actin A3*) expressed in the midgut (Bao et al., 2009) and 8 genes (*gloverin-1*, *gloverin-2*, *gloverin-3*, *gloverin-4*, *hsp19.5*, *hsp70*, *hsp90*, and *HOP*) expressed in fat body and haemocyte (Bao et al., 2010) of the KN (NB) resistant strain that were induced significantly by BmNPV compared to the uninfected resistant strain. Microarray data showed that expression levels of *amino acid transporter*, *Bm122* and *glycoside hydrolases* were higher in the resistant strain than in the susceptible strain after BmNPV infection (Zhou et al., 2013). Comparative proteomic analysis showed that caspase-1 and serine protease were expressed only in the resistant strain (Qin et al., 2012). Related studies used *B. mori*-derived cell lines (Sagisaka et al., 2010; Xue et al., 2012). Microarray analysis revealed that expression of 13 genes increased significantly and 7 genes decreased significantly after BmNPV infection (Sagisaka et al., 2010). Transcriptome analysis showed that the cytoskeleton, transcription, translation, energy metabolism, iron ion metabolism and the ubiquitin-proteasome pathway were altered after BmNPV

infection (Xue et al., 2012). However, further study is needed to determine if these genes have antiviral effects. If a single gene is primarily responsible for the high level of resistance seen in the KN (NB) strain, positional cloning would be a better method.

The silkworm midgut is an important immune organ and is the first line of resistance against pathogens. Antiviral proteins including Bmlipase-1 (Ponnuvel et al., 2003), BmSP-2 (Nakazawa et al., 2004; Yao et al., 2008), BmNOX (Selot et al., 2010, 2007) and red fluorescent proteins (RFPs) (Funakoshi and Aizawa, 1989; Sunagar et al., 2011) have been isolated from silkworm larvae gut juice. *Bmlipase-1*, which is hormonally regulated and not influenced by infection with BmNPV, is highly expressed in the anterior and middle portions of the silkworm midgut (Ponnuvel et al., 2003). Mature Bmlipase-1 is a 29 kDa protein with strong antiviral activity against BmNPV. ODVs (860 ng/larva) incubated with Bmlipase-1 (2.2 μg/larva) did not kill fifth instar silkworm larvae but untreated ODVs did (Ponnuvel et al., 2003). BmSP-2, a hormonally regulated 24 kDa protein, also has antiviral effects against BmNPV. *BmSP-2* is expressed in the entire midgut of silkworm larvae and its expression in not reduced after BmNPV infection (Nakazawa et al., 2004). The anti-BmNPV activity of SP-2 from Chinese wild type silkworms is 1.6-fold higher than the activity of SP-2 from domesticated silkworm (Yao et al., 2008). BmNOX levels are higher in the gut juice of silkworm larvae that have higher resistance to BmNPV (Selot et al., 2007). BmNOX is expressed mainly in the posterior portions of the midgut (Selot et al., 2010). RFP protein levels are also higher in BmNPV-resistant silkworms compared to susceptible silkworms (Sunagar et al., 2011) and BmNPV can be inactivated by RFP (Funakoshi and Aizawa, 1989). Light is thought to be essential for the RFP synthesis (Sunagar et al., 2011). Details of the antiviral mechanisms of these genes and gene products are not known and require further research.

3. The process of resistance to BmNPV in the transgenic silkworm

3.1. Inhibition of BmNPV at initial infection stage by Bmlipase-1 overexpression

Bmlipase-1 has antiviral activity against BmNPV at the initial infection site (Ponnuvel et al., 2003), which was overexpressed by the BmNPV IE1 promoter (IE1P) in transgenic silkworm LI-A (Jiang et al., 2012c). Quantitative real time PCR (qRT-PCR) showed that *Bmlipase-1* mRNA level in the midgut of LI-A silkworms at the fourth instar stage is 27.3% higher than in nontransgenic control silkworms (Jiang et al., 2012c). Bmlipase-1 is synthesized in the midgut and secreted into gut juice (Ponnuvel et al., 2003). Western blots revealed that Bmlipase-1 levels are higher in the gut juice of LI-A silkworms than in controls (Jin et al., 2012). The viral DNA content in LI-A silkworms is three-fold lower than in controls and the survival rate of LI-A silkworms after oral infection with BmNPV with 10^6 occlusion bodies/larva is 33% higher than controls (Jiang et al., 2012c). No obvious difference can be seen in larvae weight or cocoon quality between LI-A and control silkworms (Jiang et al., 2012b). These results suggested that overexpression of *Bmlipase-1* increases BmNPV resistance significantly but does not affect the economic characteristics of the transgenic silkworm.

The ODV envelope is a lipid membrane with five highly conserved *per os* infectivity factors (PIF1–4 and 74) that are essential for oral infection on the surface (Fang et al., 2009; Faulkner et al., 1997; Kikhno et al., 2002; Ohkawa et al., 2005; Pijlman et al., 2003). PIF1–3 and 74 form a complex with an essential function in the initial stages of infection (Peng et al., 2010). Bmlipase-1 has lipase hydrolysis activity (Ponnuvel et al., 2003) that might destroy the ODV envelope and change the conformation of the PIF complex, preventing ODV from binding and invading midgut cells. Increased Bmlipase-1 might reduce the number of ODVs that successfully invade, which could increase silkworm survival.

3.2. Suppression of BmNPV mRNA with viral gene RNAi

The BmNPV genome (T3 strain) contains 136 genes, which include essential and nonessential genes (Gomi et al., 1999). Temporal expression of NPV genes is in immediate early, delayed early, late, and very late phases (Huh and Weaver, 1990; Jiang et al., 2013d). BmNPV contains five homologous regions (hrs) (Gomi et al., 1999), which consist of repeated sequences (Gomi et al., 1999; Guarino et al., 1986; Guarino and Summers, 1986b) and the origin of DNA replication (Kool et al., 1993; Morris and Miller, 1992) in the baculovirus genomes. Some hrs act as enhancers of NPV promoters (Carson et al., 1991; Guarino et al., 1986; Guarino and Summers, 1986a, b; Jiang et al., 2012a; Nissen and Friesen, 1989; Rodems and Friesen, 1993) and some enhance the activity of nonviral promoters (Lu et al., 1997; Viswanathan et al., 2003; Wang et al., 2013). The activity of affected promoters is further increased under the transactivation of NPV IE1 protein (Gong and Guarino, 1994; Guarino et al., 1986; Jiang et al., 2012a), which binds to palindromic 28 bp repeats of hrs (Guarino and Dong, 1994; Lin et al., 2010; Lu and Carstens, 1993; Pullen and Friesen, 1995; Rodems et al., 1997).

RNAi knocks down viral genes by targeting and destroying specific mRNAs and is an effective method of disease control. Proliferation of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is inhibited by transient double-stranded RNA (dsRNA) of *gp64* and *ie-1* (Valdes et al., 2003). Silencing *ie-1* increases resistance to BmNPV (Kanginakudru et al., 2007), however, RNAi against *lef-1* does not decrease mortality (Isobe et al., 2004) in transgenic silkworms infected with BmNPV. These results indicate that several factors affect antiviral characteristics of transgenic RNAi silkworms. These factors are assumed to include the efficiency of target genes and promoters, and the connection pattern of gene fragments and spacers. The Xia group (Jiang et al., 2013b, 2013d) targeted the essential immediate early gene *ie-1* (Taggart et al., 2012; Yamada et al., 2002), the essential delayed early gene *helicase* (Kamita and Maeda, 1993, 1997), the essential late genes *gp64* (Monsma et al., 1996) and *vp39* (Thiem and Miller, 1989), and the nonessential immediate early gene *ie-2* (Prikhod'ko et al., 1999). After BmNPV infection of the transgenic silkworms, mortalities and viral mRNA and DNA were analyzed. The results showed that, for BmNPV resistance, silencing an essential gene is better than silencing a nonessential gene (Jiang et al., 2013b); an immediate early gene is the best target for RNAi; hr3 combined with IE1P was better than IE1P and *B. mori* A4 promoter (A4P); and a "head-to-head" connection pattern was better than a "tail-to-tail" configuration (Jiang et al., 2013d). The Nagaraju and Couble groups showed that targeting multiple BmNPV genes conferred greater protection against BmNPV than targeting a viral gene in transgenic silkworms (Subbaiah et al., 2013). The economic characteristics were not affected, although resistance was enhanced significantly in transgenic RNAi silkworms (Jiang et al., 2013b,d). These results suggested that inhibition of BmNPV at the mRNA level is feasible using RNAi.

3.3. Inhibition of BmNPV protein synthesis by overexpression of hycu-ep32

The multiplication of BmNPV in BmN-4 cells is inhibited by *Hyphantria cunea* NPV (HycuNPV), which causes global protein synthesis shutdown. However, *hycu-ep32*-defective HycuNPV (vHycuΔep32) does not inhibit specific protein synthesis in co-infected BmN-4 cells. These results indicate that BmNPV

proliferation is suppressed by *hycu-ep32* by inducing global protein synthesis shutdown (Shirata et al., 2010). *Hycu-ep32* is an early, nonessential gene encoding a polypeptide of 312 amino acids with no characteristic domains or motifs (Shirata et al., 2010). *B. mori* has no homologue of *hycu-ep32* (Duan et al., 2010; Shirata et al., 2010; Xia et al., 2004).

To test whether *hycu-ep32* inhibited BmNPV multiplication in the silkworm, transgenic silkworms that overexpressed exogenous *hycu-ep32* was constructed (Jiang et al., 2012a). Protein synthesis by BmN-4 cells and BmNPV was suppressed by *hycu-ep32* (Shirata et al., 2010), suggesting that exogenous *hycu-ep32* might affect silkworm protein synthesis. A suitable promoter was needed to drive *hycu-ep32* expression, so *hycu-ep32* was overexpressed in transgenic silkworms using four promoters (Jiang et al., 2012a): the inducible promoter 39KP (Guarino and Summers, 1986a), the constitutive promoter A4P, 39KP+hr3, and A4P+hr3. Expression of *hycu-ep32* was moderate and silkworms were physically normal but *hycu-ep32* expression increased significantly as BmNPV content increased in transgenic silkworms with the 39KP+hr3 promoter (HEKG-B) (Jiang et al., 2012a). The economic characteristics of HEKG-B silkworms were unchanged compared with nontransgenic silkworms, suggesting normal physiological activity of the silkworm was not affected by *hycu-ep32*. These results might be because *hycu-ep32* expression was low or because *hycu-ep32* did not suppress protein synthesis in individual HEKG-B silkworms. The antiviral capacity of HEKG-B silkworms was increased significantly compared with nontransgenic silkworms, suggesting that inhibition of BmNPV protein synthesis is a promising area for future research (Jiang et al., 2012a; Shirata et al., 2010).

3.4. Suppression of BmNPV through regulation of host immunity

Some host signaling pathways are important in NPV infection (Katsuma et al., 2007; Xiao et al., 2009). BmNPV activates host MAPK signal pathways for efficient replication. When ERK and JNK, which are activated at late-stage infection, were suppressed by specific inhibitors or by RNAi, virus yield was reduced significantly (Katsuma et al., 2007). The Xia group cloned *BmSpry*, which is upstream of ERK and JNK in *B. mori*. Overexpression of *BmSpry* inhibits BmNPV proliferation in BmE cell line (Jin et al., unpublished data). The PI3K-Akt pathway is also required for efficient NPV infection. PI3K is activated at an early stage after AcMNPV infection, and PI3K activation is required for Akt phosphorylation. Inhibition of PI3K-Akt activation significantly reduces viral production (Xiao et al., 2009).

In sections, invading pathogens are recognized by pattern recognition receptors (PRRs). Recognition is the first step in the host response and activates immune pathways (Hoffmann, 2003; Medzhitov and Janeway, 2000). Peptidoglycan recognition protein (PGRP) is a major PRR and is well studied in *B. mori* (Tanaka et al., 2008; Yoshida et al., 1996). In *B.* mori, the Toll, Imd, JNK and JAK/STAT pathways, which are involved in immunity, respond to pathogens (Tanaka et al., 2008). When *BmPGRP2*, which was cloned after bioinformatics analysis, is knocked down by RNAi, it activates immune pathways after BmNPV infection. *BmPRGP2* knockdown enhances BmNPV resistance in transgenic silkworms (Jiang et al., unpublished data). These results indicate that regulation of a host immune pathway might inhibit BmNPV proliferation.

4. The future of antiviral strategies in transgenic *B. mori*

4.1. Optimizing and integrating anti-BmNPV strategies

Overexpression of antiviral genes and RNAi that targets viral genes using transgenic technology are two effective antiviral strategies. Jiang et al. (Jiang et al., 2013c) demonstrated that combining the two methods further enhances host resistance. The transgenic silkworm strain SW-H is the first transgenic animal to suppress a virus at multiple stages of infection. In SW-H, *Bmlipase-1* is controlled by the *B. mori* midgut-specific, high-activity promoter P2 (Jiang et al., 2013a) and dsRNA for the tandem BmNPV essential genes *ie-1*, *gp64*, *lef-1*, *lef-2* and *dnapol* is driven by hr3+IE1P (Jiang et al., 2013c). The expression level of *Bmlipase-1* in the midgut of SW-H silkworms is higher than in former transgenic silkworm LI-A (Jiang et al., 2012c), and the viral mRNA content is lower in strain SW-H than in transgenic RNAi silkworms that targeting a single viral gene *ie-1* or *gp64* (Jiang et al., 2013d), further increasing the resistance of strain SW-H. Combining the four antiviral strategies of overexpression of *Bmlipase-1*, silence of multiple viral genes, overexpression of *hycu-ep32*, and RNAi of *BmPGRP2* might create a transgenic silkworm with high resistance to BmNPV, that inhibits BmNPV at initial infection and affects virus mRNA, viral protein synthesis, and host immunity.

4.2. Possible improvement to anti-BmNPV silkworm strains

Viral proteins bind to host receptors during BmNPV infection, allowing the virus to invade host cells via membrane fusion (Horton and Burand, 1993; Keddie et al., 1989). The host receptor for BmNPV, however, has not been identified. BmNPV might have more than one receptor and receptors might form a complex that facilitates recognition and invasion of BmNPV. Moreover, the BmNPV receptor might be essential for silkworms, so knocking it out would cause lethality. By contrast, regulating a presumed primary gene that is responsible for high BmNPV resistance by transgenic technology would increase the antiviral capacity of the silkworm. Receptor and primary resistance genes are interesting targets for making antiviral transgenic silkworms and worthy of further study. Several crystal structures of NPV genes have been characterized (Hou et al., 2012; Ji et al., 2010; Kadlec et al., 2008). A small molecular substrate designed to target the active region of the viral protein might block the proliferation of BmNPV.

4.3. Transgenic silkworms with enhanced resistance to other viruses

BmDNV, single-strand DNA virus (Bando et al., 1995), and BmCPV, a dsRNA virus (Hill et al., 1999), are major silkworm pathogens. Unlike BmNPV, however, BmDNV and BmCPV infect only the silkworm midgut. Some silkworm strains are resistant to BmDNV at any viral dose. Resistance to BmDNV-1 (Ina isolate) is determined by genes *nsd-1* and *Nid-1*, which block early and late infection steps, respectively (Kidokoro et al., 2010). The *nsd-2* mutation is a 6 kb deletion in the open reading frame of $+^{nsd\text{-}2}$, that results in resistance to BmDNV-2 (Saku isolate and Yamanashi isolate) (Ito et al., 2008). Nonsusceptibility to BmDNV-3 (China isolate) is caused by *nsd-Z*, which is located on chromosome 15 (Li et al., 2006). No silkworm strain is reported to have absolute resistance to BmCPV, however, and no confirmed gene inhibits BmCPV infection. The infection mechanism of BmDNV and BmCPV and the molecular mechanism underlying the host cell response to these two viruses are unclear. Knockout of *nsd-2* might generate a transgenic silkworm with resistance to BmDNV-2. However, whether *nsd-2* affects other isolates of BmDNV is unknown. Therefore, another strategy to generate transgenic silkworms with viral resistance is to use RNAi against multiple genes, specifically targeting sequences common to all BmDNV isolates. The strategy currently used to enhance resistance to BmCPV is to silence multiple viral genes under the control of a midgut-specific promoter (Jiang et al., 2013a). Transcriptome sequencing and metabolomics

might identify factors that inhibit all BmDNV isolates and help identify candidate genes for BmCPV resistance.

4.4. Construction of transgenic silkworms with resistance to multiple viruses

BmNPV, BmCPV and BmDNV all cause serious sericulture losses but BmNPV is the primary threat to silkworms. To address this challenge, future studies should construct transgenic silkworms with high resistance to multiple viruses. Currently, commercial silkworm strains grown in almost all countries are generated by two generations of hybridization involving four different parents: two parents provide economic characteristics and two parents provide disease resistance. An ideal antiviral strategy would select two parents with desirable economic traits for transgenic improvement; for example, one parent for generating transgenic silkworms with a high level of BmNPV resistance and another parent for improved resistance to BmCPV and BmDNV. Compared to nontransgenic hybrid silkworms, which have four parents, hybrids with two transgenic parents might have powerful resistance to multiple viruses and better economic characteristics, including shortened breeding time.

5. Problems in commercial application of transgenic silkworms

Two major challenges must be solved before transgenic antiviral silkworms become commercially available: (1) selection of silkworm strains for transgenic improvement, and (2) security assessment of transgenic silkworms. Silkworm strains used in sericulture are different from strains used in the laboratory for genetic improvement. Sericulture strains have been selected for greater silk production and robust adaptability to a range of environmental conditions. Strains with excellent economic traits and moderate disease resistance would be the best candidates for transgenic antiviral improvement.

Security assessment of transgenic silkworms is mainly the molecular detection of inserted genes and the impact of transgenic silkworms on the environment and human health. Potential problems that must be analyzed in the security assessment are if: (1) inserted genes are stably inherited and expressed as expected in transgenic silkworms; (2) viability and competitiveness of transgenic silkworms in the wild is changed compared to nontransgenic silkworms; (3) transgenic silkworms affect nontarget organisms; (4) transgenic silkworms affect biodiversity; or (5) inserted genes in transgenic silkworms are toxic or cause allergies in humans. As the only truly domesticated insect, *B. mori* is completely dependent on humans for survival and reproduction (Goldsmith et al., 2005). All life cycle stages of *B. mori* must be indoors, the larvae eat only mulberry leaves (*Morus* species), and the moths cannot fly. No safety problems have been recorded in the long history of sericulture, so transgenic silkworms are theoretically safe for the environment. *B. mori* strain SW-H is being tested for safety (Jiang et al., 2013c) and is likely to be the first commercially available transgenic animal with antiviral traits.

6. Conclusion

Crossbreeding techniques for *B. mori* first appeared in the early of 20th century and led to a significant increase in sericulture production capacity. Improving silkworm production capacity is difficult using traditional breeding methods; many shortcomings have been identified during a century of experiments. Transgenic silkworms can be generated that are unchanged in economic characteristics but have significantly increased viral resistance (Jiang et al., 2012a,b,c, 2013b,c,d). Thus, problems associated with traditional breeding methods can be overcome using transgenic technology. Use of transgenic antiviral silkworms to decrease silkworm larvae mortality would provide new strains for sericulture. Studies of anti-BmNPV methods that target multiple stages of pathogen infection could pave the way for disease and pest control in other organisms.

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